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AMRL-TDR-63-20

URINARY CATECHOLAMINE RESPONSE TO WATER IMMERSION

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TECHNICAL DOCUMENTARY REPORT NO. AMRL-TDR-63-20

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Project 7222, Task 722201

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FOREWORD

This work was prepared under Project 7222, "Biophysics of Flight," Task 722201, "Psychophysiology of Flight." The work was done in the Psychophysiological Stress Branch, Multi-Environment Division, Biophysics Laboratory, 6570th Aerospace Medical Research Laboratories, between May 1962 and November 1962. The catecholamine determinations were performed by Dr. McChesney Goodall, University of Tennessee, under USAF Contract AF 33(616)-7324.

ABSTRACT

The urinary excretion of adrenaline and noradrenaline was measured by bioassay for 16 normal human subjects during 6 hours of complete water immersion. The excretion of adrenaline was moderately increased, possibly related to the anxiety associated with the immersion. The excretion of noradrenaline was significantly ($p < 0.01$) reduced during immersion. Six subjects were studied during passive vertical tilt following immersion. Orthostatic intolerance was demonstrated and the increase in pulse rate and decrease in pulse pressure were significantly different from the control tilt. The probable mechanisms of the reduced noradrenaline excretion during immersion and its relation to the postimmersion impairment of orthostatic tolerance are discussed.

PUBLICATION REVIEW

This technical documentary report has been reviewed and approved.

Jos M Quashnock

JOS. M. QUASHNOCK
Colonel, USAF, MC
Chief, Biophysics Laboratory

URINARY CATECHOLAMINE RESPONSE TO WATER IMMERSION

INTRODUCTION

Man in normal gravity is primarily upright and an efficient system of reflex cardiovascular mechanisms has evolved which compensates for the hydrostatic pressure effects of body fluids due to gravity. When one stands erect most of the large arteries and veins are oriented parallel to the gravitational field and significant hydrostatic pressures are produced in these long columns of blood. Within 20 minutes of quiet standing (ref. 1), 400 to 600 cc of plasma may be pooled in the extracellular space of the legs. The circulatory mechanisms that compensate for the dependent pooling of blood and maintain venous return and cardiac output include: the tissue pressure of the extremities, the muscle pump of the legs, the abdominothoracic pump mechanisms, the return of unabsorbed capillary filtrate by the lymphatics, and reflex vasoconstriction and cardioacceleration (ref. 1). If for some reason these compensatory mechanisms are insufficient or are delayed, orthostatic hypotension and syncope may result.

The normal human subject when passively tilted erect (70°-90°) responds with a moderate increase in heart rate, a slight rise in diastolic blood pressure, a slight fall in systolic blood pressure, a decrease in pulse pressure and, occasionally, syncope (ref. 1). Orthostatic intolerance with an increased heart rate and decreased pulse pressure on the tilt table, relative to control tilt, is a consequence of prolonged bed rest (ref. 2), and more recently has been demonstrated after exposure of human subjects to complete water immersion for 6 to 12 hours (ref. 3). The physiologic mechanisms responsible for the loss of orthostatic tolerance following bed rest and water immersion are unknown, but may be related to diminished blood volume, decreased muscle or tissue pressure in the extremities, or functional alterations in the sympathetic nervous system.

Noradrenaline is the neurohormone of sympathetic (adrenergic) nerves and is released from adrenergic nerve endings (ref. 4). Although noradrenaline is also found in the adrenal medulla, adrenaline is, in the human, the chief medullary hormone (ref. 4). The release of adrenaline and noradrenaline is reflected in the urinary output of both free and conjugated forms of the two hormones (ref. 4).

The increased vasomotor activity during standing, evidenced by the increase in heart rate and the rise in diastolic blood pressure, is accompanied by an increased endogenous production of noradrenaline. Serum levels (refs. 5, 6) and urinary output of noradrenaline (ref. 7) are increased during quiet standing or vertical tilt. This increased production and output of noradrenaline are assumed to be due to activation of the vasomotor system as it occurs in bilaterally adrenalectomized subjects (ref. 8).

To ascertain the role of the sympathetic nervous system in the impairment of orthostatic tolerance following water immersion, the urinary catecholamine response of 16 subjects to 6 hours of complete water immersion was studied and related to subsequent orthostatic tolerance on the tilt table.

METHODS

The water-immersion facility was a steel tank (7 by 7 by 9 feet) lined with fiberglas internally for rust protection. Large windows in two sides permitted observation of the subject. The water temperature was thermostatically controlled at 33° C. A filter system connected to a pump cleaned the water on demand. The subjects wore a modified rubber skin-diving suit and had no skin contact with the water (figure 1). A urine relief receptacle drained through a polyethylene tube through the tank wall, enabling the subjects to void at will. Filtered air was delivered to a modified partial pressure helmet and exhausted from the helmet through a regulator which compensated breathing pressure for ambient water pressure. The immersion runs began at 0800 and continued for 6 hours. The subjects had unrestricted activity in this water-filled "room." The only instructions were to "do anything you want but remain primarily upright." The resulting buoyancy of each subject was such that he was able to regulate his depth entirely by controlling his chest size.

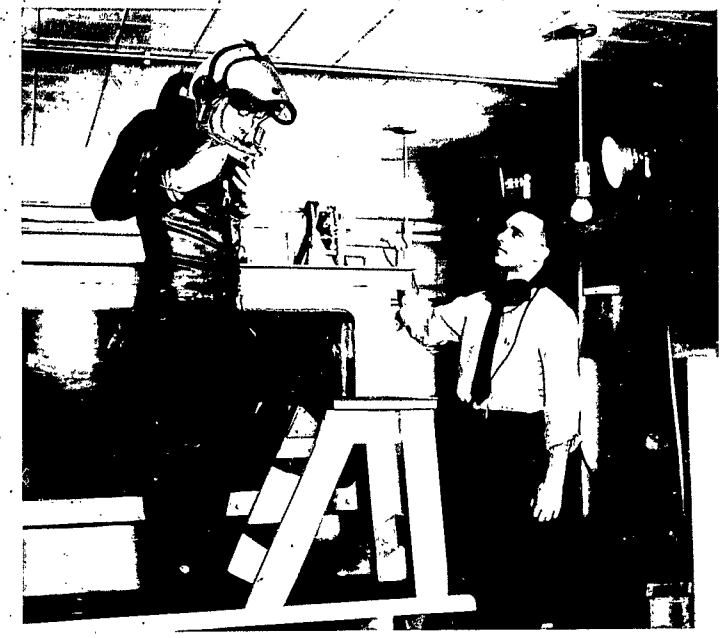

Figure 1. Medical Officer Wearing the Intercommunication Headset and a Subject with Rubber Suit, Gloves, and Helmet in Place, in Front of the Tank Prior to Immersion

The 16 subjects used in this study were healthy young men between the ages of 22 and 32. All had prior experience in the immersion tank. Subjects were told to avoid strenuous activity, get a normal night's sleep, and report to the tank room after their usual breakfast without coffee. At 0800 the subjects voided and entered the tank. A 6-hour urine sample was then obtained, its volume and specific gravity noted, and a 100-cc aliquot acidified to a pH between 2.0 and 3.0 with $2NH_2SO_4$ and frozen for subsequent catecholamine determination. On the subject's control day the same schedule was followed except that 6 hours of normal office activity was substituted for immersion. One immersion and two control urines were collected from each subject.

The urine sample was extracted by the method of von Euler and Hellner (ref. 9) and adrenaline and noradrenaline were measured by bioassay. The blood pressure of cats, sensitive to noradrenaline, and the rectal cecum of fowls, sensitive to adrenaline, were the bioassay techniques used. These techniques have been described in detail (refs. 9, 10). The various fluorometric methods now available for the determination of adrenaline and noradrenaline are not completely satisfactory because urine contains many known and unknown materials which are fluorometrically active and are therefore a source of error in the method. For these reasons and because bioassay measures the biological activity of the naturally occurring isomers of adrenaline and noradrenaline, the bioassay was used in preference to the fluorometric method.

The heart rate and blood pressure response to passive vertical tilt (70°) was determined for 6 of the 16 subjects following immersion and following a 6-hour control period. The subjects left the tank unassisted, sat in a chair while the immersion gear was removed (approximately 5 minutes), and then mounted the tilt table where they lay recumbent until a stable heart rate and blood pressure were obtained (approximately 8 minutes). Heart rate was continuously measured in beats per minute from Lead I of a conventional four-limb lead electrocardiogram during the tilt. Blood pressure was taken by auscultation with a standard clinical sphygmomanometer, the last audible sound being considered as diastole. Blood pressure was measured every minute during the 10-minute tilt.

RESULTS

All 16 subjects excreted increased amounts of dilute urine during immersion. The rate of urine flow during immersion was approximately four times the control rate (table I). The characteristics and probable mechanisms of this immersion diuresis have been described in detail (ref. 11). The urinary adrenaline and noradrenaline were calculated on the basis of an estimated 24-hour urine output and are expressed in micrograms per hour. The catecholamine levels are therefore not related to the rate of urine flow.

Two 6-hour-control urines and one 6-hour-immersion urine were collected from each of 16 subjects. One of the controls of each of four subjects (Nos. 5, 6, 10, and 13) was inadvertently destroyed during the catecholamine determination. The mean rates of catecholamine excretion are presented for 28 control and 16 immersion urines (table I and figure 2). The mean urinary excretion of adrenaline during the control period was 0.65 ± 0.06 (Standard Error - SE) μg/hour. During immersion the mean excretion rate was 0.95 ± 0.13 μg/hour, but the observed increase is not significantly different ($p < 0.15 > 0.10$). The mean excretion rate of noradrenaline during the control period was 2.50 ± 0.18 μg/hour, while the immersion rate was 1.51 ± 0.29 μg/hour. The urinary excretion of noradrenaline during immersion is significantly less than that during the control period ($p < 0.01$).

TABLE I

URINARY OUTPUT OF ADRENALINE AND NORADRENALINE
DURING 6 HOURS OF OFFICE ROUTINE (CONTROL)
AND DURING 6 HOURS OF WATER IMMERSION

Subject No.	Urine Volume (cc/6 hours)		Adrenaline (μg/hour)		Noradrenaline (μg/hour)	
	Control	Immersion	Control	Immersion	Control	Immersion
1	218 225	2312	0.65 0.53	2.00	2.72 2.48	0.77
2	690 467	1657	0.46 0.58	1.02	1.86 1.58	0.69
3	312 323	985	1.18 0.78	0.97	1.22 2.46	0.89
4	192 215	1610	0.39 0.32	1.42	1.36 3.55	0.70
5	264	766	0.94	1.26	2.79	0.88
6	270	1120	0.51	0.63	1.09	0.54
7	225 172	810	0.95 0.37	0.31	2.87 3.84	0.30
8	144 98	244	0.61 0.94	0.31	1.90 1.79	1.37
9	382 205	1115	1.01 0.29	0.95	2.90 2.05	1.47
10	191	1650	0.31	1.02	4.20	2.48
11	142 315	775	0.29 0.56	0.36	2.11 2.47	0.94
12	329 380	1234	1.43 0.45	1.05	2.44 2.06	0.74
13	760	905	0.29	0.56	0.98	2.40
14	426 500	1412	0.36 1.03	0.71	2.83 4.77	2.85
15	234 366	1258	0.32 0.59	0.50	0.98 2.96	2.63
16	267 192	1403	1.00 1.13	2.10	4.36 3.42	4.52
Mean SE ±	304	1203	0.65 0.06	0.95 0.13	2.50 0.18	1.51 0.29
T p			1.27 <0.15 >0.10		2.95 <0.01	

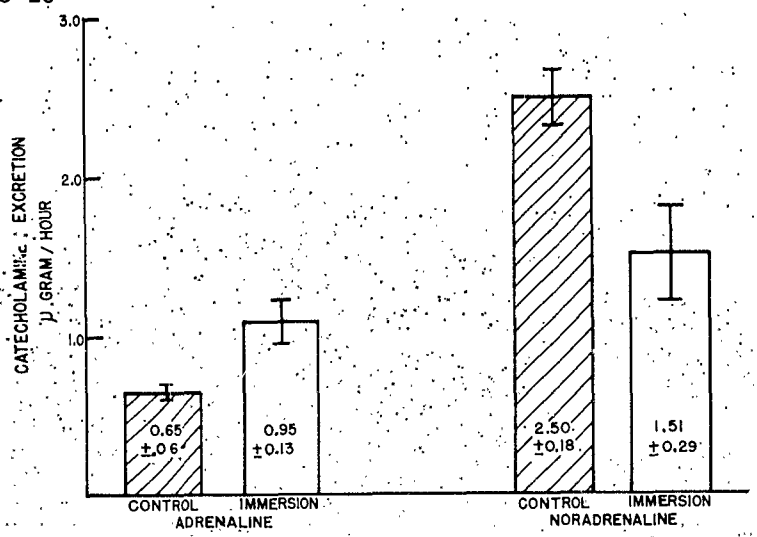

Figure 2. The Mean and Standard Error of Adrenaline and Noradrenaline Excretion during Control and Immersion Periods in Micrograms per Hour

In a single subject (no. 16) urine specimens were collected serially every 2 hours during the immersion period. The noradrenaline values were 2.25, 1.26, and 1.01 μg/hour for the first, second, and third 2-hour periods, respectively (figure 3), demonstrating a progressive decline in noradrenaline excretion during the immersion period. The subject climbed partially out of the tank to void every 2 hours requiring moderate muscular exertion.

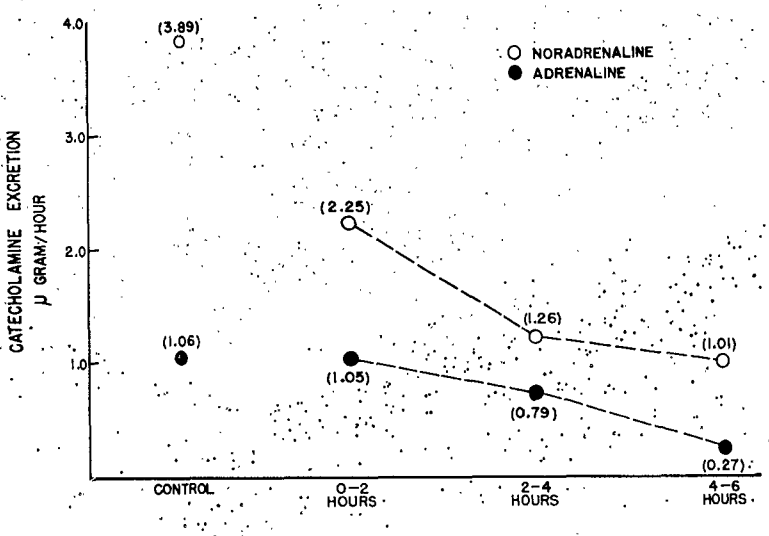

Figure 3. Adrenaline and Noradrenaline Excretion in Micrograms per Hour in a Single Subject (number 16) During a 6-Hour Control Period and During Three Consecutive 2-Hour Periods of Immersion

The heart rate and systolic and diastolic blood pressure responses during recumbency and at 2, 5, and 10 minutes after vertical tilt are given in tables II and III. Heart rate increases moderately during the control tilt from a recumbent mean of 73 beats/minute to 90, 93, and 96 at 2, 5, and 10 minutes of tilt, respectively. After immersion the heart rate increased from a recumbent mean of 66 to 97, 95, and 102 during tilt. The mean pulse rate during tilt after immersion is not significantly higher than during control tilt, at 2, 5, and 10 minutes. The systolic blood pressure fell somewhat more during tilt after immersion than during control tilt. The diastolic pressure rose somewhat more but the observed differences in the means of control and immersion tilts are not significant (tables II and III). The maximum increase in heart rate was calculated by subtracting the fastest rate recorded during the tilt, regardless of its time of occurrence, from the resting or recumbent heart rate (table IV). The mean maximum increase in heart rate after immersion is 41.5 beats/minute as compared to an increase of 27.8 beats/minute during control tilt. The difference is highly significant ($p < 0.005$, paired samples). The maximum decrease in pulse pressure was calculated similarly (table IV). The mean maximum decrease in pulse pressure is 20.1 mm Hg for the control tilt and 31.3 for the postimmersion tilt. This difference is also significant ($p < 0.05 > 0.025$, paired samples).

TABLE II

PULSE RATE IN BEATS/MINUTE, AND SYSTOLIC AND DIASTOLIC BLOOD PRESSURES IN mm Hg OF 6 SUBJECTS ON THE TILT TABLE AFTER 6 HOURS OF COMPLETE WATER IMMERSION

	Recumbent			Tilted								
				After 2 Minutes			After 5 Minutes			After 10 Minutes		
Subject Number	Pulse Rate	Blood Pressure		Pulse Rate	Blood Pressure		Pulse Rate	Blood Pressure		Pulse Rate	Blood Pressure	
		Systolic	Diastolic		Systolic	Diastolic		Systolic	Diastolic		Systolic	Diastolic
1	60	155	92	78	136	102	82	155	102	86	148	106
2	72	120	70	92	118	87	103	108	94	97	119	88
3	57	124	64	110	116	98	114	118	100	109	120	98
4	72	112	74	108	110	82	96	110	80	107	110	90
5	68	126	80	99	116	90	68	90	60	*—	—	—
6	58	128	58	96	116	64	104	116	68	114	101	70
Mean	66	128	73	97	119	87	95	116	84	102	119	90
SE (±)	2.8	6.0	4.9	4.8	3.6	5.5	6.8	8.7	7.1	5.5	6.4	4.9

*Subject fainted at sixth minute.

TABLE III

PULSE RATE IN BEATS/MINUTE, AND SYSTOLIC AND DIASTOLIC BLOOD PRESSURE IN mm Hg OF 6 SUBJECTS ON THE TILT TABLE AFTER 6 HOURS OF OFFICE ACTIVITY CONTROL

Subject Number	Recumbent			Tilted								
				After 2 Minutes			After 5 Minutes			After 10 Minutes		
	Pulse Rate	Blood Pressure		Pulse Rate	Blood Pressure		Pulse Rate	Blood Pressure		Pulse Rate	Blood Pressure	
		Systolic	Diastolic		Systolic	Diastolic		Systolic	Diastolic		Systolic	Diastolic
1	61	154	86	67	146	90	63	151	98	65	152	96
2	80	112	70	98	110	78	96	108	75	106	110	74
3	56	112	76	84	114	90	93	120	88	100	128	86
4	68	109	78	86	114	86	91	118	90	88	110	82
5	91	136	78	103	134	84	101	131	80	108	134	78
6	79	140	58	101	135	62	113	125	64	112	120	80
Mean	73	127	74	90	126	82	93	125	83	96	126	83
SE (±)	5.4	7.6	3.9	5.6	7.9	4.3	6.7	6.0	4.9	7.2	6.4	3.1

TABLE IV

THE MAXIMUM INCREASE IN PULSE RATE IN BEATS/MINUTE AND THE MAXIMUM DECREASE IN PULSE PRESSURE IN mm Hg DURING TILT OF 6 SUBJECTS FOLLOWING 6-HOUR-CONTROL AND 6-HOUR-IMMERSION PERIODS

Subject No.	Maximum Change in Pulse Pressure		Maximum Change in Pulse Rate	
	Control	Immersion	Control	Immersion
1	16	29	10	29
2	10	36	26	31
3	18	44	52	61
4	15	20	24	40
5	10	20	22	32
6	42	39	33	56
Mean	20.1	31.3	27.8	41.5
SE(±)	4.5	4.1	5.7	5.6
n	6	6	6	6
T (paired samples)	2.16		4.91	
p	<0.05>0.025		<0.005	

One subject (no. 14) fainted after 6 minutes of the postimmersion tilt (figure 4). Pallor, bradycardia, restlessness, hyperpnea, nausea, and dimming of vision were noted over a minute's time. Not one subject fainted during the control tilts. The heart rate and blood pressure responses to control and postimmersion tilt of another subject (no. 12) are presented to show the characteristic alterations in orthostatic tolerance following water immersion (figure 5).

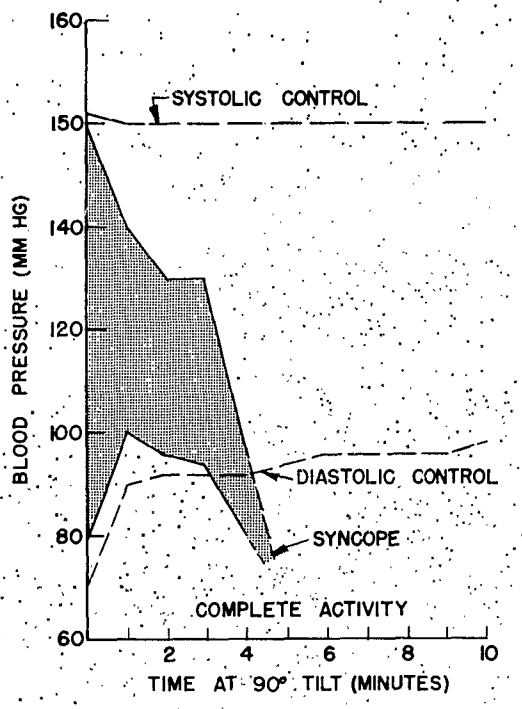

Figure 4. Blood Pressure Response (shaded area) to Passive Vertical Tilt in a Single Subject (number 14) after 6 Hours of Water Immersion Demonstrating Changes from Control Systolic (upper dotted line) and Control Diastolic Pressures (bottom dotted lines) with Syncope at the Fifth Minute

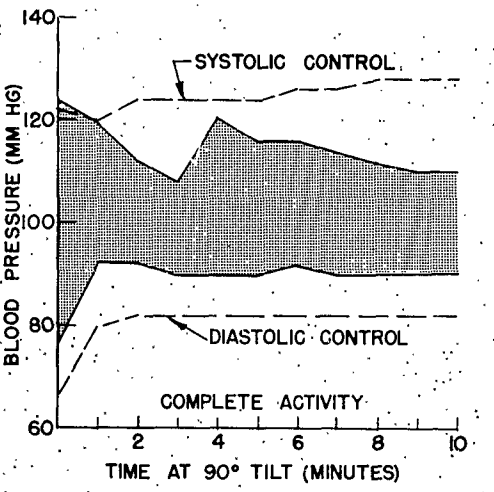

Figure 5. Typical Blood Pressure Response to Passive Vertical Tilt after 6 Hours of Immersion (shaded area) Compared to Control Pressures

DISCUSSION

The data presented confirm the apparent decrease in orthostatic tolerance following water immersion that has been previously described (ref. 3). The blood pressure and heart rate responses to tilt after immersion are significantly different from controls when tested as paired samples. One subject fainted on the tilt table following immersion while none fainted during control tilt. The incidence of fainting in normal subjects tilted for 20 minutes has been described as approximately 10 (ref. 12) and 20 (ref. 13) percent.

The urinary excretion of adrenaline is increased during immersion, although not significantly. This increase is most likely related to the emotion or anxiety associated with the immersion procedure. In one subject (no. 16) the adrenaline content of the urine for the last 2 hours of immersion was only 25% of that present in the first 2-hour specimen (figure 3). Goodall and Berman have shown that during the stress of acceleration an increased urinary excretion of adrenaline was related to the attendant anticipation and anxiety and not to the stress itself (ref. 14). The increase in urinary adrenaline observed at the same time noradrenaline excretion is decreased demonstrates the independent release of these hormones, previously demonstrated in anxiety (ref. 14) and hypothalamic stimulation (ref. 4).

Noradrenaline excretion is significantly decreased during 6 hours of water immersion. The noradrenaline output of human subjects is slightly less during bed rest than during daily activity (ref. 4) and increases in an exponential fashion with increasing muscular work (ref. 5). Sundin has shown that normal subjects excrete considerably larger amounts of noradrenaline when tilted to 75 degrees in comparison with the excretion in the recumbent position (ref. 7). The increased noradrenaline excretion during standing and during exercise is probably related to the associated increase in vasomotor activity. Conversely, the diminished noradrenaline excretion of water immersion may be related to diminished vasomotor activity, as in the water-immersion situation both the level of muscular activity and the cardiovascular compensations necessary for changes in posture are minimized (ref. 3).

The relation of the decreased output of noradrenaline during water immersion to the subsequent impairment of tilt table tolerance remains to be determined by further study of the sympathetico-adrenal response to tilt following immersion.

In a weightless environment, there will be no hydrostatic pressure effects due to gravity, and therefore no demand on reflex sympathetic compensation. Analogous situations occur in bed rest where hydrostatic pressure effects are minimized by the horizontal position and in water immersion where hydrostatic pressures are neutralized by ambient water pressure. Because of prolonged disuse, reflex vasomotor responses may become less efficient in the weightless state and intolerance to acceleration and orthostasis may result (ref. 3). The observations that noradrenaline excretion is reduced during prolonged bed rest and during prolonged water immersion support this suggestion.

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3. Urine
4. Orthostatic Tolerance
I. AFSC Project 7222, Task 722201
II. Biophysics Laboratory
III. McCally, M., M.C., Graveline, D.E., M.C.
IV. In ASTIA collection
V. Aval fr OTS: $0.50

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noradrenaline was significantly ($p < 0.01$) reduced during immersion. Six subjects were studied during passive vertical tilt following immersion. Orthostatic intolerance was demonstrated and the increase in pulse rate and decrease in pulse pressure were significantly different from the control tilt. The probable mechanisms of the reduced noradrenaline excretion during immersion and its relation to the postimmersion impairment of orthostatic tolerance are discussed.

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